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(54) **Nucleic acid-bound polypeptide, method of producing nucleic acid-bound polypetide, and immuoassay using the polypeptide**

(57) A nucleic acid-bound polypeptide produced by binding a nucleic acid to a polypeptide, a method of producing the nucleic acid-bound polypeptide, and applications of the nucleic acid-bound polypeptide, including immunoassays for an antigen or antibody, such as an agglutination immunoassay are provided.

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a nucleic acid-bound polypeptide, a method of producing the nucleic acid-bound polypeptide, and an immunoassay using the nucleic acid-bound polypeptide.

Discussion of Background

Various studies have been made as to how to maintain the specific steric structure of a recombinant protein produced by gene engineering, more specifically gene manipulation, and also as to how to apply the thus produced protein to an antigen-antibody reaction.

In the production of the recombinant protein, in particular, in the course of a purification step of the produced protein, a denaturation operation is inevitably carried out. In such purification step, it is not always possible to maintain a natural structure of the protein, so that such protein cannot be used in an immunoassay system.

Various factors are also known that affect reactions which are peculiar to each of various assays. It is known that for these reasons or other, the above-mentioned antigen-antibody reaction does not always proceed as desired when the recombinant protein is used.

For example, there is known an agglutination immunoassay as one of immunoassays. For instance, when an antibody corresponding to an antigen is assayed by agglutination immunoassay, the antigen is fixed on the surface of particles such as latex particles, and such antigen-fixed particles are allowed to react with the antibody in a test sample. When the antibody is present in the test sample, the antigen-fixed particles agglutinate due to the antigen-antibody reaction, so that, for instance, the absorbance of the test sample changes. Therefore by measuring the absorbance of the test sample, the degree of the agglutination can be determined, and accordingly the antibody in the test sample can be quantitatively measured from the measured absorbance of the test sample.

However, when the recombinant protein is used as the antigen to be fixed on the surface of the particles in the above-mentioned agglutination immunoassay, it occasionally occurs that even though the protein itself has reactivity with the antibody to be assayed and the antibody is in fact present in the test sample, no agglutination takes place.

Conventionally, in the case where no agglutination takes place as mentioned above, the recombinant protein is modified or expressed in the form of a fused protein in order to improve the agglutination reactivity of the protein. However, it is extremely difficult to modify the protein so as to impart the desired properties thereto, while maintaining the antigenicity (i.e. the reactivity with the antibody).

Furthermore, the recombinant protein is often of an insoluble kind, so that when the thus produced protein is purified, the protein has to be subjected to solubilization treatment. However, the protein is often denatured in the course of the purification treatment, losing the necessary antigenicity.

Therefore, it is preferable that a soluble protein be directly produced by genetic engineering.

SUMMARY OF THE INVENTION

It is therefore a first object of the present invention to provide a modified polypeptide, which is modified so as to change the properties of polypeptide such as the isoelectric point, the molecular weight and the three-dimensional structure thereof, but without changing the antigenicity thereof.

A second object of the present invention is to provide a method of producing the above-mentioned recombinant polypeptide in such a manner that the produced polypeptide can be obtained in a soluble fraction.

A third object of the present invention is to provide an immunoassay for assaying an antigen comprising a polypeptide, which is conventionally difficult to perform.

The first object of the present invention can be achieved by a nucleic acid-bound polypeptide which is obtainable by binding a nucleic acid to a polypeptide.

In the above nucleic acid-bound polypeptide, the nucleic acid may be bound to at least one terminus of the polypeptide.

The nucleic acid-bound polypeptide may further comprise a nucleic acid-binding motif through which the nucleic acid is bound to the polypeptide.

The above-mentioned polypeptide and the nucleic acid-binding motif may be expressed in the form of a fusion polypeptide by genetic engineering.

The nucleic acid-binding motif may have an amino acid sequence with sequence No. 2 defined in a sequence table attached to the specification of this application.

The above-mentioned polypeptide can be used as an antigen to be assayed by an immunoassay.

The second object of the present invention can be achieved by a method of producing a nucleic acid-bound polypeptide comprising the steps of:

producing a recombinant polypeptide,
binding a nucleic acid to the recombinant polypeptide to produce a nucleic acid-bound polypeptide as a soluble fraction, and
purifying the nucleic acid-bound polypeptide from the soluble fraction.

In the above-mentioned method of producing the nucleic acid-bound polypeptide, the step of binding the nucleic acid to the polypeptide to produce the nucleic acid-bound polypeptide may comprise the steps of:

fusing a gene which encodes the polypeptide and a gene which encodes the nucleic acid-binding motif to produce a fusion gene, and
expressing the fusion gene to produce the nucleic acid-bound polypeptide via the nucleic acid-binding motif.

The third object of the present invention can be achieved by an immunoassay for assaying an antigen comprising a polypeptide, or an antibody corresponding to the antigen, using as the antigen the above-mentioned nucleic acid-bound polypeptide, obtainable by binding a nucleic acid to said polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Fig. 1 is a genetic map of a cloning vector pW6A for use in expressing HCV core protein used in the examples of the present invention.

Fig. 2 is a diagram showing the results of Western blotting performed for showing the reactivity of an HCV core protein prepared by genetic engineering in an example of the present invention with HCV core positive human serum.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The nucleic acid-bound polypeptide of the present invention can be provided by binding a nucleic acid to a polypeptide, whereby the properties of the polypeptide, such as the isoelectric point, the molecular weight and the three-dimensional structure thereof, can be changed without the antigenicity thereof being changed.

As the "polypeptide" for use in the present invention, any polypeptide can be employed as long as the polypeptide itself exhibits antigenicity and therefore the number of amino acid residues which constitute the polypeptide is 6 or more. It is preferable that the number of the amino acid residues which constitute the "polypeptide" for use in the present invention be 8 or more.

Examples of the "polypeptide" for use in the present invention include composites of a polypeptide and another component or other components such as sugar or lipid, namely glycoprotein and lipoprotein.

There is no particular limitation to the size of the nucleic acid which is bound to the polypeptide as long as the nucleic acid can change the above-mentioned properties of the polypeptide, such as isoelectric point, molecular weight and three-dimensional structure, without changing the antigenicity thereof. Normally, the number of bases of the nucleic acid for use in the present invention is 100 b to 10 kb, preferably about 1 kb to 5 kb.

Furthermore, the nucleic acid to be bound to the polypeptide may be either DNA or RNA. In the present invention, there is no limitation to the nucleotide sequence to be bound to the polypeptide. Any nucleotide sequence is acceptable for use in the present invention.

The nucleic acid may be bonded to any portion of the polypeptide. For instance, the nucleic acid may be bonded to the N-terminus or the C-terminus of the polypeptide, but the bonding is not limited to such terminus. In the present invention, the nucleic acid may be either directly or indirectly bonded to the polypeptide. For instance, the nucleic acid may be bonded to the polypeptide via a nucleic acid-binding motif which is also a polypeptide.

In this application, with respect to the binding of the nucleic acid to the polypeptide, the term "binding" or "bound" means all kinds of chemical bondings between the polypeptide and the nucleic acid with attractive force in a wide range of relatively weak attractive force to strong attractive force, without any particular limitation to the bonding mode, including the so-called association, covalent bonding, ionic bonding, coordinate bonding, and hydrogen bonding.

In the present invention, when the nucleic acid-bound polypeptide is produced by genetic engineering, the nucleic acid-bound polypeptide may be expressed in the form of a polypeptide to which the nucleic acid is bound, thereby

pW6AHCV core 120 was prepared.

By use of this plasmid, *Escherichia coli* BL21 (DE3) (obtained from Brookhaven National Laboratory) was subjected to transformation, so that an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120 for expressing HCV core polypeptide 120 was obtained, and an HCV core protein (1 - 120 aa) was expressed. Hereinafter, the thus expressed protein is referred to as "120". The nucleotide sequence of "120" and the amino acid sequence of "120" are respectively shown in Sequence ID. No. 3 and Sequence ID. No. 4 in the sequence table attached to this specification.

Example 1

[Preparation of Plasmid]

A DNA fragment for coding HCV core polypeptides 150 and 120 which are respectively shown with sequence ID. No. 5 and with sequence ID. No. 3 in the attached sequence table was amplified by the PCR (Polymerase Chain Reaction) method, using as a template molecule a plasmid CKSC1150 with a DNA fragment including an HCV core region being introduced, and was then digested with a restriction endonuclease EcoRI and a restriction endonuclease BamHI.

An HCV core region-including DNA fragment 470 bp and an HCV core region-including DNA fragment 370 bp were separated by 1% agarose gel electrophoresis. These DNA fragments were inserted into an EcoRI - BamHI site of the expression plasmid pW6A shown in Fig. 1, whereby a plasmid pW6AHCV core 150 and a plasmid pW6AHCV core 120 were prepared.

A DNA fragment for coding an HBc nucleic acid-binding motif shown with sequence ID. No. 1 in the sequence table attached to this specification was amplified by the PCR (Polymerase Chain Reaction) method, using as a template molecule a plasmid pHBV-11 (Nucleic Acids Res., 18, 4587 (1990)), and was then digested with the BamHI restriction endonuclease.

A DNA fragment 110bp including a nucleic acid-binding motif was separated by 2% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI - BamHI site of each of the above-mentioned plasmid pW6AHCV core 150 and plasmid pW6AHCV core 120.

By use of these plasmids, *Escherichia coli* BL21 (DE3) (obtained from Brookhaven National Laboratory) was subjected to transformation, so that an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 150NA and an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA were obtained.

In this specification, the proteins to which the nucleic acid-binding motif is bound for expressing the above-mentioned transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 150NA and the above-mentioned transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA are respectively referred to as "150NA" and "120NA".

The nucleotide sequence of "150NA" and the amino acid sequence of "150NA" are respectively shown in Sequence ID. No. 9 and Sequence ID. No. 10 in the sequence table attached to this specification; and the nucleotide sequence of "120NA" and the amino acid sequence of "120NA" are respectively shown in Sequence ID. No. 7 and Sequence ID. No. 8 in the sequence table attached to this specification.

Example 2

[Expression of Recombinant Protein (150NA and 120NA)]

Each of the transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 150 and the transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120 prepared in Example 1 was separately cultured overnight in 2 ml of an LB culture medium containing 50 µg/ml of ampicillin at 37°C.

After the optical density (OD) of each culture medium reached 0.6 to 0.8 with a light with a wavelength of 600 nm by preculture, expression induction was carried out with the addition of 0.5 mM IPTG (Isopropyl-β-D(-)-thiogalactopyranoside) thereto, and the cultivation was continued for another two hours.

1.5 ml of the *Escherichia coli* cultivation medium was centrifuged at 5000 rpm for 2 minutes, whereby the *Escherichia coli* was collected. The thus collected *Escherichia coli* was suspended in 100 µl of a buffer solution (10 mM tris-HCl, pH 8.0, 0.1 M NaCl, 1 mM EDTA), and was then subjected to ultrasonic disruption for 15 minutes, whereby the *Escherichia coli* was completely disrupted, whereby two test samples, namely an *Escherichia coli* test sample of *Escherichia coli* BL21 (DE3)/pW6AHCV core 150NA and an *Escherichia coli* test sample of *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA, were prepared.

8 µl of a three-fold concentrated SDS polyacrylamide buffer solution (0.15 M tris-HCl, pH 6.8, 6% SDS, 24% glycerol, 6 mM EDTA, 2% 2-mercaptoethanol, 0.003% bromophenol blue) was added to each of the above test samples separately. Each mixture was then stirred sufficiently and was subjected to SDS-polyacrylamide gel electrophoresis.

producing the nucleic acid-bound polypeptide. Alternatively, after a recombinant polypeptide is expressed, the nucleic acid may be bound to the recombinant polypeptide, thereby producing the nucleic acid-bound polypeptide.

To be more specific, when a polypeptide is expressed as a fusion polypeptide, with a nucleic acid-binding motif which is known to have a function of binding the nucleic acid to the polypeptide being included in the function of the polypeptide to be expressed, a polypeptide with the nucleic acid-binding motif is expressed, and the nucleic acid in the host is simultaneously bound to the recombinant polypeptide via the nucleic acid-binding motif, so that the nucleic acid-bound polypeptide can be produced. This nucleic acid-bound polypeptide can be purified thereafter.

Alternatively, the nucleic acid-bound polypeptide can be obtained by reconstituting the polypeptide by mixing the expressed polypeptide with the nucleic acid.

In connection with the above-mentioned nucleic acid-binding motif, various nucleic acid-binding motifs are known. For example, in J. of Virology, 64 3319-3330 (1990), there is reported a nucleic acid-binding motif which is present in HBc protein amino acid sequence of hepatitis B virus (HBV), and in Biochim. Biophys. Act, 950, 45-53 (1988), there is reported protamin, which is a nucleic acid-bound protein in mouse. These can also be employed in the present invention.

The nucleotide sequence and the amino acid sequence of the nucleic acid-binding motif of HBc are respectively shown in the sequence No. 1 and the sequence No. 2 in the sequence table attached to this specification; and the nucleotide sequence and the amino acid sequence of the mouse protamin are respectively shown in the sequence No. 17 and the sequence No. 18 in the sequence table attached to this specification

As mentioned above, when the protein or polypeptide conventionally produced by genetic engineering is used as the antigen to be fixed on the surface of the particles in the conventional agglutination immunoassay, it occasionally occurs that even though the polypeptide itself has reactivity with the antibody to be assayed and the antibody is in fact present in the test sample, no agglutination takes place.

In the present invention, however, this conventional problem is completely solved by use of the nucleic acid-bound polypeptide. Namely, when the nucleic acid-bound polypeptide of the present invention is used as the antigen to be fixed on the surface of particles for use in the agglutination immunoassay, the agglutination successfully takes place proportionally in accordance with the amount of the corresponding antibody in the test sample.

The nucleic acid-bound polypeptide of the present invention can be applied not only to the above-mentioned agglutination, but also to any conventional immunoassay such as ELISA (enzyme-linked immunosorbent assay).

Furthermore, the polypeptide antigen in a test sample can also be assayed by carrying out a competition reaction with the addition of a known amount of the nucleic acid-bound polypeptide to the test sample.

Conventionally, when a polypeptide is produced by genetic engineering, in many cases, the recombinant polypeptide is obtained as an insoluble fraction. Therefore, when the thus obtained polypeptide is used in practice, the polypeptide must be subjected to solubilization treatment. However, the polypeptide is often denatured in the course of the solubilization treatment, changing the antigenicity. Therefore it is preferable that the recombinant polypeptide be obtained as a soluble fraction.

In the method of producing the nucleic acid-bound polypeptide of the present invention, for example, a polypeptide is produced by genetic engineering, and the thus produced polypeptide is simultaneously caused to be bound to a nucleic acid in the host, whereby the nucleic acid-bound polypeptide is obtained as a soluble fraction.

Furthermore, as shown in the following examples, for example, when the polypeptide to be expressed is expressed as a fused polypeptide of a polypeptide and a nucleic acid-binding motif of HBc, the nucleic acid is bound to the nucleic acid-binding motif at the same time as the expression thereof, so that the nucleic acid-bound polypeptide is obtained in the soluble fraction.

Thus, there can be attained the method of producing the nucleic acid-bound polypeptide of the present invention, which comprises the steps of producing the recombinant polypeptide, binding the nucleic acid to the polypeptide to produce the nucleic acid-bound polypeptide as a soluble fraction, and purifying the nucleic acid-bound polypeptide from the soluble fraction. Other features of this invention will become apparent in the course of the following description of exemplary embodiments, which are given for illustration of the invention and are not intended to be limiting thereof.

Reference Example 1

[Expression of HCV Core Protein (1 - 120aa)]

A DNA fragment for coding the HCV core polypeptide with sequence ID. No. 3 in the attached sequence table was amplified by the PCR (Polymerase Chain Reaction) method, using as a template molecule a plasmid CKSC1150 with a DNA fragment including an HCV core region, and was then digested with a restriction endonuclease EcoRI and a restriction endonuclease BamHI.

An HCV core region-including DNA fragment 370 bp was separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI - BamHI site of an expression plasmid pW6A shown in Fig. 1, so that a plasmid

Western blotting was performed on a nitrocellulose filter, using each of the thus prepared test samples. After performing blocking using 1% BSA, each of the test samples was allowed to react with an HCV core antibody human serum which was diluted 200 times with a phosphoric acid buffer solution (10 mM phosphoric acid, pH 7.4, 0.15 M NaCl). Furthermore, a peroxydase enzyme labeled anti-human IgG rabbit polyclonal antibody (made by Daco Co., Ltd.) was then allowed to react therewith. After washing, 10 ml of a substrate coloring liquid (0.01 % aqueous solution of hydrogen peroxide, 0.6 mg/ml 4-chloro-1-naphthol) was added thereto, whereby each test sample was colored.

The results are shown in Fig. 2. As shown in Fig. 2, both the *Escherichia coli* test sample of *Escherichia coli* BL21 (DE3)/pW6AHCV core 150NA and the *Escherichia coli* test sample of *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA exhibited a positive reaction with the HCV core antibody human serum.

Example 3

[Purification of Soluble Nucleic Acid-bound 120NA Recombinant Protein (120(+))]

The *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA prepared in Example 1 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to be about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and thereafter the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.2 M NaCl, 0.3% octylthioglucoside (hereinafter referred to as "OTG") were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby a soluble fraction which contained therein a nucleic acid-bound 120NA (hereinafter referred to as "120NA(+)") was recovered.

A 50%-sucrose concentration buffer solution was prepared by adding sugar to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 30%-sucrose concentration buffer solution, and a 20%-sucrose concentration buffer solution were prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution, the 30%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The 120NA(+) containing soluble fraction was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a first sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using a Beckman ultrasonic centrifuge.

The 120NA(+) was recovered in a portion with a sucrose concentration of about 30 to 40%.

The 120NA(+) containing fraction recovered by the first sucrose density gradient ultracentrifugation was purified by Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (0.3 M NaCl, 0.1% myristyl sulfobetaine (Trademark "SB3-14" made by Sigma Co., Ltd.), whereby 120NA(+) with a molecular weight of about 700 to 1000 kDa was recovered.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 20%-sucrose concentration buffer solution was prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The above-mentioned 120NA(+) with a molecular weight of about 700 to 1000 kDa was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a second sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using the Beckman ultrasonic centrifuge, whereby the 120NA(+) was concentrated and purified.

Reference Example 2

[Purification of Insoluble 120NA]

The *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA prepared in Example 1 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to have about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was then centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.2 M NaCl, 0.3% OTG) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby an insoluble 120NA fraction was obtained. The thus obtained insoluble 120NA fraction was made soluble by a buffer solution (6M urea, 50 mM glycine-NaOH, pH 11.7) and was then subjected to centrifugation, whereby a supernatant fraction was obtained.

The thus obtained supernatant fraction was subjected to ion exchange purification, using an SFF cationic ion exchange column (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea, 50 mM glycine-NaOH, pH 11.7), with sodium chloride elution.

The SFF eluted fraction was then purified, using Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-0.5M NaOH, 50 mM tris-HCl, pH 9.6). Thus, a purified 120NA was obtained in a portion with a molecular weight of about 22 kDa.

Example 4

[Confirmation of Properties of 120NA and 120NA (+)]

The OD 260/280 nm ratio of the 120NA(+) purified in Example 3 was measured. The result was that the OD 260/280 nm ratio of the 120NA(+) was about 2.0, which was greater than the OD 260/280 nm ratio of the 120NA. This indicated that at least the polypeptide and the nucleic acid coexist in the 120NA(+).

Furthermore, in the sucrose density gradient ultracentrifugation, the 120NA was mostly collected in the zero% sucrose concentration region, while the 120NA(+) was mostly collected in an about 30-40% sucrose concentration region. It is considered that this fact indicates that the density of the 120NA(+) is different from that of the 120NA.

The 120NA(+) was subjected to enzyme treatment, using DNase or RNase. When the 120NA(+) was subjected to enzyme treatment, using RNase, the nucleic acid contained in the 120NA(+) was decomposed in its entirety by the RNase. It is considered that this fact indicates that the constituent nucleic acid of the 120NA(+) is RNA.

The 120NA(+) was also subjected to isoelectric focusing. The isoelectric point of the 120NA(+) was present in a wide range of pI 3.5 to 5.0.

In sharp contrast to this, the isoelectric point of the 120NA purified in Reference Example 2 was pI 12.84, with a strong positive charge, which was significantly different from the isoelectric point of the 120NA(+).

Furthermore, the 120NA(+) was also subjected to Native electrophoresis, using a 3% agarose 3% polyacrylamide gel. From the fact that luminescence was observed at the time of Ethidium bromide stain of the 120NA(+), it was confirmed that the nucleic acid was contained in the 120NA(+).

The 120NA(+) was further subjected to Western blotting and Coomassie Brilliant Blue stain, using the same gel as used in the above-mentioned Ethidium Bromide stain. The result was that in the Western blotting, the reactivity of the 120NA(+) with an anti-HCV core antibody was observed at the same position as that of the portion made luminescent by the Ethidium Bromide stain; and in the Coomassie Brilliant Blue stain, the presence of the polypeptide was confirmed.

In sharp contrast to this, with respect to the 120A, the transfer of the 120NA into the gel was not confirmed in the Native electrophoresis even when the Western blotting and the Coomassie Brilliant Blue stain were carried out.

Thus, the properties of the 120NA(+) are entirely different from those of the 120NA with respect to the apparent molecular weight, the density, and the electric charge thereof, particularly because of the increase of the apparent molecular weight of the 120NA(+) due to the binding of the nucleic acid to the polypeptide in the 120NA(+), but there are no differences in the Western blotting and agglutination reactions between the two. From these facts, it is considered that the antigenicity is maintained in the 120NA(+).

Reference Example 3

[Expression of Lysine-fused 120 (120K10)]

In the same manner as in Example 1, pW6AHCV core 120 was subjected to such gene manipulation that 10 lysine residues were continuously fused to the C-terminus of pW6AHCV core 120, whereby pW6AHCV core 120K10 was prepared.

By use of this pW6AHCV core 120K10, *Escherichia coli* BL21 (DE3) was subjected to transformation, whereby an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core K10 was obtained. Hereinafter, the protein expressed by this ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core K10 is referred to as 120K10.

The above transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core K10 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to have about 0.7

when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium then was centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli,* 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.2 M NaCl, 0.3% OTG) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby a soluble 120K10 fraction and an insoluble 120K10 fraction were separately obtained.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 30%-sucrose concentration buffer solution and a 20%-sucrose concentration buffer solution were prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution, the 30%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The above-mentioned soluble 120K10 fraction was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using the Beckman ultrasonic centrifuge. The 120K10 was not recovered in any of the 50%-sucrose concentration buffer solution, the 30%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution, but was recovered on the top layer portion in the tube.

The above-mentioned insoluble 120K10 fraction was purified in the same manner as in Reference Example 2, using the SFF cationic ion exchange column (made by Pharmacia Co., Ltd.) and performing the gel filtration, whereby a purified 120K10 was recovered in a portion with a molecular weight of about 20 kDa.

Reference Example 4

[Assay of HCV Core Antigen Positive Serum]

The reactivity of each of HCV antibody positive serum 1 and HCV antibody positive serum 2 with a commercially available HCV antibody assay agent (Trademark "RIBA HCV 3.0 STRIP IMMUNOBLOT ASSAY" made by Chiron Co., Ltd.) was tested, using HCV antigen c100 (Amino acid Nos. 1569-1931), HCV antigen c33c (Amino acid Nos. 1192-1457), core antigen c22 (Amino acid Nos. 2-120) and NS5 (Amino acid Nos. 2054-2995). The result was that both HCV antibody positive serum 1 and HCV antibody positive serum 2 have antibodies in the entire antigen region including the core antigen region.

TABLE 1

Reactivity Tests of Positive Serums					
	c100	c33c	Core Antigen	NS5	Judgement
Positive Serum 1	4+	4+	4+	4+	Positive
Positive Serum 2	4+	4+	4+	4+	Positive

Example 5

Each of the HCV antigens obtained in Reference Examples 1, 2, 3 and Example 3 was fixed on the surface of gelatin particles (made by Fujirebio Co., Ltd.) with a concentration of 10 mg/ml in a buffer solution (0.15M PBS, pH 7.1).

By use of HCV antibody positive serum 1 and HCV antibody positive serum 2 confirmed as having antibodies in the entire antigen region including the core antigen region in Reference Example 4, and a monoclonal antibody #2-7 obtained by subjecting HCV core antigen c22 to immunization, the immune reactivity of each of the above-mentioned HCV antigens fixed on the surface of gelatin particles was investigated.

25 µl of each HCV antigen-fixed gelatin particle and 25 µl of one of the above-mentioned HCV antibody positive serum 1 or HCV antibody positive serum 2, or 25 µl of the monoclonal antibody #2-7 were allowed to react in a microtiter plate (made by Fujirebio Co., Ltd.) for 2 hours, and agglutination images thereof were investigated. The results are shown in TABLE 2. In TABLE 2, the reactivity is shown with a dilution rate of 2^n, and when a positive agglutination image was observed even when n was 4 or more in the dilution rate, the immune reactivity was judged as being "positive".

The monoclonal antibody #2-7 obtained by subjecting HCV core antigen c22 to immunization reacted with any

HCV core antigen, but it was only with the 120NA(+) fixed gelatin particles that HCV antibody positive serum 1 and HCV antibody positive serum 2 reacted in the above-mentioned reactions.

TABLE 2

Immune Reactivity Tests of HCV Core Antigens			
Name of Core Antigen	Positive Serum 1	Positive Serum 2	#2-7
120NA(+)	6+	7	8
120NA	<3	<3	7
120K10	<3	<3	6
120	<3	<3	4

Example 6

[Rearrangement of 120NA(+) from 120NA]

By use of the transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA prepared in Example 1, HCV core 120NA was purified from an insoluble fraction thereof in the same manner as in Reference Example 2. The molecular weight of the purified HCV core 120NA was about 22 kDa, and the OD 260/280 nm ratio thereof was about 0.7.

To the HCV core 120NA (hereinafter referred to as 120NA), a cyclic plasmid DNA (4.7 Kbp) derived from pW6A, 6M urea and 20% sucrose were added, and 120NA was dialyzed against a buffer solution (50 mM tris-HCL, 0.15M NaCl, 20% sucrose), whereby 120NA was rearranged to 120NA(+).

The 120NA(+) which was obtained by the above-mentioned dialysis and rearrangement was purified, using Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.), whereby the 120NA(+) was recovered in a portion with a molecular weight of 700 to 1000 KD.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 20%-sucrose concentration buffer solution was prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The above recovered 120NA(+) was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using the Beckman ultrasonic centrifuge. The rearranged 120NA(+) was recovered in an about 40% to 50%-sucrose concentration portion of the buffer solution.

The OD 260/280 nm ratio of the 120NA before the rearrangement was about 0.7, and when the 120NA was rearranged to the 120NA(+), the OD 260/280 nm ratio thereof was changed from about 0.7 to about 1.7.

Furthermore, the above-mentioned rearranged 120NA(+) and the soluble 120NA(+) prepared in Example 3 have almost the same molecular weight after the gel filtration thereof, and also have almost the same specific weight thereof after the sucrose density gradient ultracentrifugation thereof. Thus, it is considered that these facts indicate that the above-mentioned rearrangement from the 120NA to the 120NA(+) was successfully conducted.

Example 7

[Construction of Transformed *Escherichia coli* BL21 (DE3)/pW6AHCV Core 120NA120 for Expressing 120-fused 120NA (120NA120)]

A DNA fragment for coding an HCV core polypeptide shown with sequence ID. No. 3 in the attached sequence table was amplified by the PCR method, using as a template molecule a plasmid CKSC1150 with a DNA fragment including an HCV core region being introduced, and was then digested with a restriction endonuclease NheI and a restriction endonuclease EcoRI.

An HCV core region-including DNA fragment 370 bp was separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into an NheI - EcoRI site of the expression plasmid pW6A shown in Fig. 1, whereby a plasmid pW6AHCV core 120 (NheI/EcoRI) was prepared.

A DNA fragment for coding the HCV core polypeptide shown with sequence ID. No. 3 in the attached sequence table was amplified by the PCR method, using as a template molecule a plasmid CKSC1150, and was then digested with a restriction endonuclease EcoRI and a restriction endonuclease BamHI.

An HCV core region-including DNA fragment 370 bp was then separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI - BamHI site of the plasmid pW6AHCV core 120 (NheI/EcoRI), whereby a plasmid pW6AHCV core 120-120 was prepared.

A DNA fragment for coding an HBc nucleic acid-binding motif with sequence ID. No. 1 in the attached sequence table was amplified by the PCR method, using as a template molecule a plasmid pHBV-11, and was then digested with a restriction endonuclease EcoRI.

A DNA fragment 110bp including the nucleic acid-binding motif was separated by 2% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI site of the above-mentioned plasmid pW6AHCV core 120-120.

By use of this plasmid, *Escherichia coli* BL21 (DE3) was subjected to transformation, so that an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA120 for expressing 120-fused 120NA (hereinafter referred to as 120NA120) was obtained.

Example 8

[Purification of Insoluble 120NA120]

In the same manner as in Reference Example 2, the transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA120 prepared in Example 7 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to have about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was then centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.2 M NaCl, 0.3% OTG) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby an expressed 120NA120 was obtained as a soluble fraction as well as an insoluble fraction. The insoluble 120NA120 fraction was made soluble by a buffer solution (6M urea, 50 mM glycine-NaOH, pH 11.0) and was then subjected to centrifugation, whereby a supernatant fraction was obtained.

The thus obtained supernatant fraction was subjected to ion exchange purification, using an SFF cationic ion exchange column (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-glycine-NaOH, pH 11.0), with sodium chloride elution. 120NA120 was recovered in an about 0.5M sodium chloride elution fraction.

The SFF eluted fraction was then purified, using Superdex 200 (gel filtration column)(made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-0.5M NaCl, 50 mM tris-HCl, pH 9.6). Thus, a purified 120NA120 was obtained in a portion with a molecular weight of about 40 kDa.

The nucleotide sequence and the amino acid sequence of the 120NA120 are respectively shown with Sequence ID. No. 11 and Sequence ID. No. 12 in the attached sequence table.

Example 9

[Purification of Soluble Nucleic Acid-bound 120NA120 (120NA120(+)]

In the same manner as in Example 3, the *Escherichia coli* BL21 (DE3)/pW6AHCV core 120NA120 prepared in Example 7 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to be about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and thereafter the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.2 M NaCl, 0.3% OTG) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby a soluble nucleic acid-bound 120NA120 (hereinafter referred to as "120NA120(+)") was recovered.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 30%-sucrose concentration buffer solution, and a 20%-sucrose concentration buffer solution were prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution, the 30%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The 120NA120(+) containing soluble fraction was overlaid on top of the overlaid buffer solutions in the ultracen-

trifuge tube, and was then subjected to a first sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using a Beckman ultrasonic centrifuge.

The 120NA120(+) was recovered in a portion with a sucrose concentration of about 30 to 40%.

The 120NA120(+) containing fraction recovered by the first sucrose density gradient ultracentrifugation was purified by Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (0.3 M NaCl, 0.3% OTG, 50 mM glycine-NaOH, pH 10.0), whereby 120NA120(+) with a molecular weight of about 700 to 1000 kDa was recovered.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 20%-sucrose concentration buffer solution was prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The above-mentioned 120NA120(+) with a molecular weight of about 700 to 1000 kDa was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a second sucrose density gradient centrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using the Beckman ultrasonic centrifuge, whereby the 120NA120(+) was concentrated and purified.

Example 10

[Rearrangement of 120NA120 to 120NA120(+)]

The OD 260/280 nm ratio of the 120NA120 purified in Example 9 was about 0.7.

To the purified 120NA120, there was added a purified DNA (about 1.3 to 0.7 Kbp)(made by Sigma Co., Ltd.), which was obtained form calf thymus and was subjected to sufficient cleavage by a restriction endonuclease Hae3. Furthermore, 6M urea, 20% sucrose and 1.0 M NaCl were added thereto.

This mixture was dialyzed against a buffer (50 mM tris-HCl, 0.3 M NaCl) at 4°C, whereby the 120NA120 was rearranged to a soluble 120NA120(+).

The soluble 120NA120(+) was purified by Superdex 200 (gel filtration column)(made by Pharmacia Co., Ltd.), whereby a purified 120NA120(+) was recovered in a portion with a molecular weight of about 700 to 1000 kDa. The OD 260/280 nm ratio of the thus recovered rearranged 120NA120(+) was about 1.8.

Example 11

[Construction of Transformed *Escherichia coli* BL21 (DE3)/pW6A47C2NA for Expressing Nucleic Acid-Binding TP47 (TP47C2NA)]

A DNA fragment encoding a 47 kDa antigen derived from TP (Treponema pallidum), with Sequence ID No. 13 in the attached sequence table, was amplified by the PCR method, using as a template molecule a plasmid pW6A47C2 with a DNA fragment including a TP 47 kDa antigen region being introduced, and was then digested with a restriction endonuclease EcoRI and a restriction endonuclease BamHI.

A TP 47 kDa antigen region-including DNA fragment 1.3 Kbp was separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI-BamHI site of the expression plasmid pW6A shown in Fig. 1, whereby a plasmid pW6A47C2(EcoRI/BamHI) was prepared.

A DNA fragment for coding an HBc nucleic acid-binding motif with Sequence ID No. 1 in the attached sequence table was amplified by the PCR method, using as a template molecule a plasmid pHBV-11, and was then digested with a restriction endonuclease HamHI and a restriction endonuclease HindIII.

A nucleic acid-binding motif-containing DNA fragment 110 bp was then separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into a BamHI-HindIII site of the above plasmid pW6A47C2 (EcoRI/BamHI).

By use of this plasmid, *Escherichia coli* BL21 (DE3) was subjected to transformation, so that an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6A47C2NA for expressing a nucleic acid-binding TP47 (hereinafter referred to as TP47C2NA) was obtained.

Example 12

[Purification of Insoluble TP47C2NA]

In the same manner as in Reference Example 2, the transformed *Escherichia coli* BL21 (DE3)/pW6ATP47C2NA

prepared in Example 11 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to have about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was then centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby an expressed TP47C2NA was obtained as a soluble fraction as well as an insoluble fraction. The insoluble TP47C2NA fraction was made soluble by a buffer solution (6M urea, 50 mM tris-HCl, pH 8.0) and was then subjected to centrifugation, whereby a supernatant fraction was obtained.

The thus obtained supernatant fraction was subjected to ion exchange purification, using an SFF cationic ion exchange column (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (8M urea, sodium acetate, pH 6.0), with sodium chloride elution. TP47C2NA was recovered in an about 0.5M sodium chloride elution fraction.

The SFF eluted fraction was then purified, using Superdex 200 (gel filtration column)(made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-0.5M NaCl, 50 mM tris-HCl, pH 9.6). Thus, a purified TP47C2NA was obtained in a portion with a molecular weight of about 100 kDa.

The nucleotide sequence and the amino acid sequence of the TP47C2NA are respectively shown with Sequence ID No. 15 and Sequence ID No. 16 in the attached sequence table.

Example 13

[Purification of Soluble Nucleic Acid-bound TP47C2NA (TP47C2NA(+)]

In the same manner as in Example 3, the *Escherichia coli* BL21 (DE3)/pW6ATP47C2NA prepared in Example 11 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to be about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and thereafter the cultivation was continued for two hours and 30 minutes.

The *Escherichia coli* cultivation medium was centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol, 0.3% OTG) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby a soluble nucleic acid-bound TP47C2NA (hereinafter referred to as "TP47C2NA(+)") was recovered.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 30%-sucrose concentration buffer solution, and a 20%-sucrose concentration buffer solution were prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution, the 30%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The TP47C2NA(+) containing soluble fraction was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a first sucrose density gradient ultracentrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using a Beckman ultrasonic centrifuge.

The TP47C2NA(+) was recovered in a portion with a sucrose concentration of about 30 to 45%.

The TP47C2NA(+) containing fraction recovered by the first sucrose density gradient ultracentrifugation was purified by Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (0.3 M NaCl, 0.3% OTG, 50 mM glycine-NaOH, pH 10.0), whereby TP47C2NA(+) was recovered in a portion with a molecular weight of about 700 to 1000 kDa.

A 50%-sucrose concentration buffer solution was prepared by adding sucrose to a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) in such a manner that the concentration of sucrose in the buffer solution was 50%. In the same manner as mentioned above, a 20%-sucrose concentration buffer solution was prepared.

These buffer solutions were overlaid in an ultracentrifuge tube in the direction from the bottom to the top portion of the tube in the order of the 50%-sucrose concentration buffer solution and the 20%-sucrose concentration buffer solution.

The above-mentioned TP47C2NA(+) with a molecular weight of about 700 to 1000 kDa was overlaid on top of the overlaid buffer solutions in the ultracentrifuge tube, and was then subjected to a second sucrose density gradient centrifugation at 10°C, with a centrifugal force of 100,000 g, for 12 hours, using the Beckman ultrasonic centrifuge,

whereby the TP47C2NA(+) was concentrated and purified.

Example 14

[Rearrangement of TP47C2NA to TP47C2NA(+)]

The OD 260/280 nm ratio of the TP47C2NA purified in Example 12 was about 0.6.

To the purified TP47C2NA, there was added a purified DNA (about 1.3 to 0.7 Kbp)(made by Sigma Co., Ltd.), which was obtained form calf thymus and was subjected to sufficient cleavage by a restriction endonuclease Hae3. Furthermore, 6M urea, 20% sucrose and 1.0 M NaCl were added thereto.

This mixture was dialyzed against a buffer (50 mM tris-HCl, 0.3 M NaCl) at 4°C, whereby the TP47C2NA was rearranged to a soluble TP47C2NA(+).

The soluble TP47C2NA(+) was purified by Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.), whereby a purified 120NA120(+) was recovered in a portion with a molecular weight of about 700 to 1000 kDa. The OD 260/280 nm ratio of the thus recovered rearranged TP47C2NA(+) was about 1.8.

Example 15

[Construction of Transformed *Escherichia coli* BL21 (DE3)/pW6ACV Core 120prol for Expressing Mouse Protamine-1 fused 120 (120prol)]

A DNA fragment for coding a mouse protamine 1 with Sequence ID No. 17 in the attached sequence table was isolated, and amplified by the PCR method, using as a template molecule a mouse protamine 1 cDNA, and was then digested with a restriction endonuclease EcoRI and a restriction endonuclease BamHI.

A mouse protamine 1 region-including DNA fragment 160 bp was separated by 1% agarose gel electrophoresis. This DNA fragment was inserted into an EcoRI-BamHI site of the plasmid pW6AHCV core 120 (NheI/EcoRI) prepared in Example 1.

By use of this plasmid, *Escherichia coli* BL21 (DE3) was subjected to transformation, so that an ampicillin-resistant transformed *Escherichia coli* BL21 (DE3)/pW6ACV core 120prol for expressing a mouse protamine 1 fused 120 (hereinafter referred to as 120prol) was obtained.

Example 16

[Purification of 120prol]

In the same manner as in Reference Example 2, the transformed *Escherichia coli* BL21 (DE3)/pW6AHCV core 120prol prepared in Example 15 was cultured overnight in an LB culture medium at 37°C. The optical density (OD) of the culture medium was adjusted by preculture so as to have about 0.7 when measured with light with a wavelength of 600 nm. Expression induction was then carried out with the addition of 0.5 mM IPTG thereto, and the cultivation was continued for 2 hours and 30 minutes.

The *Escherichia coli* cultivation medium was then centrifuged, whereby the *Escherichia coli* was collected. To the thus collected *Escherichia coli*, 150 ml of a buffer solution (50 mM tris-HCl, pH 8.0, 20% ethanol) were added, and the mixture was ice-cooled and subjected to ultrasonic disruption.

This mixture was then centrifuged, whereby an expressed 120prol was obtained as a soluble fraction as well as an insoluble fraction. The insoluble 120prol fraction was made soluble by a buffer solution (6M urea, 50 mM glycine-NaOH, pH 11.0) and was then subjected to centrifugation, whereby a supernatant fraction was obtained.

The thus obtained supernatant fraction was subjected to ion exchange purification, using an SFF cationic ion exchange column (made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-glycine-NaOH, pH 11.0), with sodium chloride elution. 120prol was recovered in an about 0.5M sodium chloride elution fraction.

The SFF eluted fraction was then purified, using Superdex 200 (gel filtration column)(made by Pharmacia Co., Ltd.) which was equilibrated with a buffer solution (6M urea-0.5M NaCl, 50 mM tris-HCl, pH 9.6). Thus, a purified 120prol was obtained in a portion with a molecular weight of about 22 kDa.

The nucleotide sequence and the amino acid sequence of the 120prol are respectively shown with Sequence ID No. 19 and Sequence ID No. 20 in the attached sequence table.

Example 17

[Rearrangement of 120prol to 120prol(+)]

The OD 260/280 nm ratio of the 120prol purified in Example 16 was about 0.7.

To the purified 120prol, there was added a purified DNA (about 1.3 to 0.7 Kbp)(made by Sigma Co., Ltd.), which was obtained form calf thymus and was subjected to sufficient cleavage by a restriction endonuclease Hae3. Furthermore, 6M urea, 20% sucrose and 1.0 M NaCl were added thereto.

This mixture was dialyzed against a buffer (50 mM tris-HCl, 0.3M NaCl) at 4°C, whereby the 120prol was rearranged to a soluble 120prol(+).

The soluble 120prol(+) was purified by Superdex 200 (gel filtration column) (made by Pharmacia Co., Ltd.), whereby a purified 120prol(+) was recovered in a portion with a molecular weight of about 700 to 1000 kDa. The OD 260/280 nm ratio of the thus recovered rearranged 120prol(+) was about 1.7.

Thus, the present invention provides the nucleic acid-bound polypeptide with various properties of the polypeptide being changed, without changing the antigenicity thereof. The use of the nucleic acid-bound polypeptide of the present invention makes it possible to perform immunoassays which have been conventionally impossible.

Furthermore, according to the present invention, there is provided a method of recovering a genetic product in a soluble fraction, which has conventionally been recovered in an insoluble fraction.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Fuminori TAKEMURA et al.

(ii) TITLE OF INVENTION: NUCLEIC ACID-BOUND POLYPEPTIDE. METHOD OF PRODUCING NUCLEIC ACID-BOUND POLYPEPTIDE. AND IMMUNOASSAY USING THE POLYPEPTIDE

(iii)NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

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(F) POSTAL CODE (ZIP): 163-07

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER:NEC PC

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE:

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

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(A) APPLICATION NUMBER: JP 134444/1996

(B) FILING DATE: 1-MAY-1996

(viii)ATTORNEY/AGENT INFORMATION

(A) NAME:

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER:

(xi) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:

(B) TELEFAX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:1 : FROM 1 to 102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1 :

AGACGACGAG GCAGGTCCCC TAGAAGAAGA ACTCCCTCGC CTCGCAGACG AAGGTCTAAA
30 60

TCGCCGCGTC GCAGAAGATC TCAATCTCGG GAATCTCAAT GT

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:2 : FROM 1 to 34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2 :

Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg
1 5 10 15

Arg Arg Ser Lys Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg Glu Ser
20 25 30

Gln Cys

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:3 : FROM 1 to 360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3 :

```
ATGAGCACAA ATCCTAAACC TCAAAGAAAA ACCAAACGTA ACACCAACCG CCGCCCACAG
1                              30                             60
GACGTTAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACCTGTT GCCGCGCAGG
                               90                            120
GGCCCCAGGT TGGGTGTGCG CGCGACTAGG AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
                              150                            180
AGGCGACAAC CTATCCCCAA GGCTCGCCGG CCCGAGGGTA GGACCTGGGC TCAGCCCGGG
                              210                            240
TACCCTTGGC CCCTCTATGG CAACGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTCACCC
                              270                            300
CGTGGCTCTC GGCCTAGTTG GGGCCCCACA GACCCCCGGC GTAGGTCGCG TAATTTGGGT
                              330                            360
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:4 : FROM 1 to 120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4 :

```
Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
1               5                   10                  15
Arg Arg Pro Arg Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
            20                  25                  30
Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
        35                  40                  45
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
    50                  55                  60
Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65                  70                  75                  80
```

Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp Ala Gly Trp
85 90 95

Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
100 105 110

Arg Arg Arg Ser Arg Asn Leu Gly
115 120

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 450 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:5 : FROM 1 to 450

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5 :

ATGAGCACAA ATCCTAAACC TCAAAGAAAA ACCAAACGTA ACACCAACCG CCGCCCACGG
30 60

GACGTTAAAT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACCTGTT GCCGCGCAGG
90 120

GGCCCCAGGT TGGGTGTGCG CGCGACTAGG AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
150 180

AGGCGACAAC CTATCCCCAA GGCTCGCCGG CCCGAGGGTA GGACCTGGGC TCAGCCCGGG
210 240

TACCCTTGGC CCCTCTATGG CAACGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTCACCC
270 300

CGTGGCTCCC GGCCTAGTTG GGGCCCCACG GACCCCCGGC GTAGGTCACG CAATTTGGGT
330 360

AAGGTCATCG ATACCCTCAC ATGCGGCTTC GCCGACCTCA TGGGGTACAT TCCGCTTGTC
390 420

GGCGCCCCCC TAGGGGGCGC TGCCAGGGCC
450

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(vi) ORIGINAL SOURCE:
(A) ORGANISM: recombinant
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Fuminori TAKEMURA et al.
(B) TITLE:
(K) RELEVANT RESIDUES IN SEQ ID NO:6 : FROM 1 to 150
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6 :

```
Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
1               5                   10                  15
Arg Arg Pro Arg Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
            20                  25                  30
Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
        35                  40                  45
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
    50                  55                  60
Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65                  70                  75                  80
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp Ala Gly Trp
                85                  90                  95
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
            100                 105                 110
Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
        115                 120                 125
Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
    130                 135                 140
Gly Gly Ala Ala Arg Ala
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 nucleic acids
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:7 : FROM 1 to 483

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7 :

ATGGCTAGCG AATTCATGAG CACAAATCCT AAACCTCAAA GAAAAACCAA ACGTAACACC
1 30 60
AACCGCCGCC CACAGGACGT TAAGTTCCCG GGCGGTGGTC AGATCGTTGG TGGAGTTTAC
90 120
CTGTTGCCGC GCAGGGGCCC CAGGTTGGGT GTGCGCGCGA CTAGGAAGAC TTCCGAGCGG
150 180
TCGCAACCTC GTGGAAGGCG ACAACCTATC CCCAAGGCTC GCCGGCCCGA GGGTAGGACC
210 240
TGGGCTCAGC CCGGGTACCC TTGGCCCCTC TATGGCAACG AGGGTATGGG GTGGGCAGGA
270 300
TGGCTCCTGT CACCCCGTGG CTCTCGGCCT AGTTGGGGCC CCACAGACCC CCGGCGTAGG
330 360
TCGCGTAATT TGGGTGGATC CAGACGACGA GGCAGGTCCC CTAGAAGAAG AACTCCCTCG
390 420
CCTCGCAGAC GAAGGTCTAA ATCGCCGCGT CGCAGAAGAT CTCAATCTCG GGAATCTCAA
450 480
TGT

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 161 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:8 : FROM 1 to 161

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8 :

Met Ala Ser Glu Phe Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr
1 5 10 15
Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly

```
                20                      25                      30
Gly Gln Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg
            35                      40                      45
Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg
        50                      55                      60
Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr
65                      70                      75                      80
Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met
                    85                      90                      95
Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp
                100                     105                     110
Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Gly Ser Arg
            115                     120                     125
Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg
        130                     135                     140
Arg Ser Lys Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg Glu Ser Gln
145                     150                     155                     160
Cys
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 573 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO: 9 : FROM 1 to 573

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9 :

```
ATGGCTAGCG AATTCATGAG CACAAATCCT AAACCTCAAA GAAAAACCAA ACGTAACACC
                                  30                          60
AACCGCCGCC CACGGGACGT TAAATTCCCG GGCGGTGGTC AGATCGTTGG TGGAGTTTAC
                                  90                         120
CTGTTGCCGC GCAGGGGCCC CAGGTTGGGT GTGCGCGCGA CTAGGAAGAC TTCCGAGCGG
                                 150                         180
TCGCAACCTC GTGGAAGGCG ACAACCTATC CCCAAGGCTC GCCGGCCCGA GGGTAGGACC
```

210 240
TGGGCTCAGC CCGGGTACCC TTGGCCCCTC TATGGCAACG AGGGTATGGG GTGGGCAGGA
270 300
TGGCTCCTGT CACCCCGTGG CTCCCGGCCT AGTTGGGGCC CCACGGACCC CCGGCGTAGG
330 360
TCACGCAATT TGGGTAAGGT CATCGATACC CTCACATGCG GCTTCGCCGA CCTCATGGGG
390 420
TACATTCCGC TTGTCGGCGC CCCCCTAGGG GGCGCTGCCA GGGCCGGATC CAGACGACGA
450 480
GGCAGGTCCC CTAGAAGAAG AACTCCCTCG CCTCGCAGAC GAAGGTCTAA ATCGCCGCGT
510 540
CGCAGAAGAT CTCAATCTCG GGAATCTCAA TGT
570

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(vi) ORIGINAL SOURCE:
(A) ORGANISM: recombinant
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Fuminori TAKEMURA et al.
(B) TITLE:
(K) RELEVANT RESIDUES IN SEQ ID NO: 10 : FROM 1 to 191
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Ala Ser Glu Phe Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr
1 5 10 15
Lys Arg Asn Thr Asn Arg Arg Pro Arg Asp Val Lys Phe Pro Gly Gly
20 25 30
Gly Gln Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg
35 40 45
Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg
50 55 60
Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr
65 70 75 80
Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met
85 90 95
Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp

100 105 110

Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile

115 120 125

Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu

130 135 140

Val Gly Ala Pro Leu Gly Gly Ala Ala Arg Ala Gly Ser Arg Arg Arg

145 150 155 160

Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser

165 170 175

Lys Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg Glu Ser Gln Cys

180 185 190

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 843 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:11 : FROM 1 to 843

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11 :

ATGGCTAGCA TGAGCACAAA TCCTAAACCT CAAAGAAAAA CCAAACGTAA CACCAACCGC

30 60

CGCCCACAGG ACGTTAAGTT CCCGGGCGGT GGTCAGATCG TTGGTGGAGT TTACCTGTTG

90 120

CCGCGCAGGG GCCCCAGGTT GGGTGTGCGC GCGACTAGGA AGACTTCCGA GCGGTCGCAA

150 180

CCTCGTGGAA GGCGACAACC TATCCCCAAG GCTCGCCGGC CCGAGGGTAG GACCTGGGCT

210 240

CAGCCCGGGT ACCCTTGGCC CCTCTATGGC AACGAGGGTA TGGGGTGGGC AGGATGGCTC

270 300

CTGTCACCCC GTGGCTCTCG GCCTAGTTGG GGCCCCACAG ACCCCCGGCG TAGGTCGCGT

330 360

AATTTGGGTG AATTCAGACG ACGAGGCAGG TCCCCTAGAA GAAGAACTCC CTCGCCTCGC

390 420

AGACGAAGGT CTAAATCGCC GCGTCGCAGA AGATCTCAAT CTCGGGAATC TCAATGTGAA

450 480

TTCATGAGCA CAAATCCTAA ACCTCAAAGA AAAACCAAAC GTAACACCAA CCGCCGCCCA

510 540

CAGGACGTTA AGTTCCCGGG CGGTGGTCAG ATCGTTGGTG GAGTTTACCT GTTGCCGCGC

570 600

AGGGGCCCCA GGTTGGGTGT GCGCGCGACT AGGAAGACTT CCGAGCGGTC GCAACCTCGT

630 660

GGAAGGCGAC AACCTATCCC CAAGGCTCGC CGGCCCGAGG GTAGGACCTG GGCTCAGCCC

690 720

GGGTACCCTT GGCCCCTCTA TGGCAACGAG GGTATGGGGT GGGCAGGATG GCTCCTGTCA

750 780

CCCCGTGGCT CTCGGCCTAG TTGGGGCCCC ACAGACCCCC GGCGTAGGTC GCGTAATTTG

810 840

GGT

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 281 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:12 : FROM 1 to 281

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12 :

Met Ala Ser Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg

1 5 10 15

Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln

20 25 30

Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly

35 40 45

Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg

50 55 60

Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala

65 70 75 80

Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp

```
                85                  90                  95
Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro
            100                 105                 110
Thr Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Glu Phe Arg Arg Arg
        115                 120                 125
Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser
    130                 135                 140
Lys Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg Glu Ser Gln Cys Glu
145                 150                 155                 160
Phe Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr
                165                 170                 175
Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val
            180                 185                 190
Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg
        195                 200                 205
Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln
    210                 215                 220
Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro
225                 230                 235                 240
Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp Ala Gly
                245                 250                 255
Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp
            260                 265                 270
Pro Arg Arg Arg Ser Arg Asn Leu Gly
        275                 280
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1245 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:13 : FROM 1 to 1245

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13 :

```
ATGGGCTCGT CTCATCATGA GACGCACTAT GGCTATGCGA CGCTAAGCTA TGCGGACTAC
                                       30                      60
TGGGCCGGGG AGTTGGGGCA GAGTAGGGAC GTGCTTTTGG CGGGTAATGC CGAGGCGGAC
                                       90                     120
CGCGCGGGGG ATCTCGACGC AGGCATGTTC GATGCAGTTT CTCGCGCAAC CCACGGGCAT
                                      150                     180
GGCGCGTTCC GTCAGCAATT TCAGTACGCG GTTGAGGTAT TGGGCGAAAA GGTTCTCTCG
                                      210                     240
AAGCAGGAGA CCGAAGACAG CAGGGGAAGA AAAAAGTGGG AGTACGAGAC TGACCCAAGC
                                      270                     300
GTTACTAAGA TGGTGCGTGC CTCTGCGTCA TTTCAGGATT TGGGAGAGGA CGGGGAGATT
                                      330                     360
AAGTTTGAAG CAGTCGAGGG TGCAGTAGCG TTGGCGGATC GCGCGAGTTC CTTCATGGTT
                                      390                     420
GACAGCGAGG AATACAAGAT TACGAACGTA AAGGTTCACG GTATGAAGTT TGTCCCAGTT
                                      450                     480
GCGGTTCCTC ATGAATTAAA AGGGATTGCA AAGGAGAAGT TTCACTTCGT GGAAGACTCC
                                      510                     540
CGCGTTACGG AGAATACCAA CGGCCTTAAG ACAATGCTCA CTGAGGATAG TTTTTCTGCA
                                      570                     600
CGTAAGGTAA GCAGCATGGA GAGCCCGCAC GACCTTGTGG TAGACACGGT GGGTACCGTC
                                      630                     660
TACCACAGCC GTTTTGGTTC GGACGCAGAG GCTTCTGTGA TGCTGAAAAG GGCTGATGGC
                                      690                     720
TCTGAGCTGT CGCACCGTGA GTTCATCGAC TATGTGATGA ACTTCAACAC GGTCCGCTAC
                                      750                     780
GACTACTACG GTGATGACGC GAGCTACACC AATCTGATGG CGAGTTATGG CACCAAGCAC
                                      810                     840
TCTGCTGACT CCTGGTGGAA GACAGGAAGA GTGCCCCGCA TTTCGTGTGG TATCAACTAT
                                      870                     900
GGGTTCGATC GGTTTAAAGG TTCAGGGCCG GGATACTACA GGCTGACTTT GATTGCGAAC
                                      930                     960
GGGTATAGGG ACGTAGTTGC TGATGTGCGC TTCCTTCCCA AGTACGAGGG GAACATCGAT
                                      990                    1020
ATTGGGTTGA AGGGGAAGGT GCTGACCATA GGGGGCGCGG ACGCGGAGAC TCTGATGGAT
                                     1050                    1080
GCTGCAGTTG ACGTGTTTGC CGATGGACAG CCTAAGCTTG TCAGCGATCA AGCGGTGAGC
                                     1110                    1140
TTGGGGCAGA ATGTCCTCTC TGCGGATTTC ACTCCCGGCA CTGAGTACAC GGTTGAGGTT
                                     1170                    1200
```

AGGTTCAAGG AATTTGGTTC TGTGCGTGCG AAGGTAGTGG CCCAG
1230

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 415 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:14 : FROM 1 to 415

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14 :

```
Met Gly Ser Ser His His Glu Thr His Tyr Gly Tyr Ala Thr Leu Ser
1               5                   10                  15
Tyr Ala Asp Tyr Trp Ala Gly Glu Leu Gly Gln Ser Arg Asp Val Leu
            20                  25                  30
Leu Ala Gly Asn Ala Glu Ala Asp Arg Ala Gly Asp Leu Asp Ala Gly
        35                  40                  45
Met Phe Asp Ala Val Ser Arg Ala Thr His Gly His Gly Ala Phe Arg
    50                  55                  60
Gln Gln Phe Gln Tyr Ala Val Glu Val Leu Gly Glu Lys Val Leu Ser
65                  70                  75                  80
Lys Gln Glu Thr Glu Asp Ser Arg Gly Arg Lys Lys Trp Glu Tyr Glu
                85                  90                  95
Thr Asp Pro Ser Val Thr Lys Met Val Arg Ala Ser Ala Ser Phe Gln
            100                 105                 110
Asp Leu Gly Glu Asp Gly Glu Ile Lys Phe Glu Ala Val Glu Gly Ala
        115                 120                 125
Val Ala Leu Ala Asp Arg Ala Ser Ser Phe Met Val Asp Ser Glu Glu
    130                 135                 140
Tyr Lys Ile Thr Asn Val Lys Val His Gly Met Lys Phe Val Pro Val
145                 150                 155                 160
Ala Val Pro His Glu Leu Lys Gly Ile Ala Lys Glu Lys Phe His Phe
                165                 170                 175
Val Glu Asp Ser Arg Val Thr Glu Asn Thr Asn Gly Leu Lys Thr Met
            180                 185                 190
```

Leu Thr Glu Asp Ser Phe Ser Ala Arg Lys Val Ser Ser Met Glu Ser
195 200 205
Pro His Asp Leu Val Val Asp Thr Val Gly Thr Val Tyr His Ser Arg
210 215 220
Phe Gly Ser Asp Ala Glu Ala Ser Val Met Leu Lys Arg Ala Asp Gly
225 230 235 240
Ser Glu Leu Ser His Arg Glu Phe Ile Asp Tyr Val Met Asn Phe Asn
245 250 255
Thr Val Arg Tyr Asp Tyr Tyr Gly Asp Asp Ala Ser Tyr Thr Asn Leu
260 265 270
Met Ala Ser Tyr Gly Thr Lys His Ser Ala Asp Ser Trp Trp Lys Thr
275 280 285
Gly Arg Val Pro Arg Ile Ser Cys Gly Ile Asn Tyr Gly Phe Asp Arg
290 295 300
Phe Lys Gly Ser Gly Pro Gly Tyr Tyr Arg Leu Thr Leu Ile Ala Asn
305 310 315 320
Gly Tyr Arg Asp Val Val Ala Asp Val Arg Phe Leu Pro Lys Tyr Glu
325 330 335
Gly Asn Ile Asp Ile Gly Leu Lys Gly Lys Val Leu Thr Ile Gly Gly
340 345 350
Ala Asp Ala Glu Thr Leu Met Asp Ala Ala Val Asp Val Phe Ala Asp
355 360 365
Gly Gln Pro Lys Leu Val Ser Asp Gln Ala Val Ser Leu Gly Gln Asn
370 375 380
Val Leu Ser Ala Asp Phe Thr Pro Gly Thr Glu Tyr Thr Val Glu Val
385 390 395 400
Arg Phe Lys Glu Phe Gly Ser Val Arg Ala Lys Val Val Ala Gln
405 410 415

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1368 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:15 : FROM 1 to 1368

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15 :

```
ATGGCTAGCG AATTCATGGG CTCGTCTCAT CATGAGACGC ACTATGGCTA TGCGACGCTA
                             30                            60
AGCTATGCGG ACTACTGGGC CGGGGAGTTG GGGCAGAGTA GGGACGTGCT TTTGGCGGGT
                             90                           120
AATGCCGAGG CGGACCGCGC GGGGGATCTC GACGCAGGCA TGTTCGATGC AGTTTCTCGC
                            150                           180
GCAACCCACG GGCATGGCGC GTTCCGTCAG CAATTTCAGT ACGCGGTTGA GGTATTGGGC
                            210                           240
GAAAAGGTTC TCTCGAAGCA GGAGACCGAA GACAGCAGGG GAAGAAAAAA GTGGGAGTAC
                            270                           300
GAGACTGACC CAAGCGTTAC TAAGATGGTG CGTGCCTCTG CGTCATTTCA GGATTTGGGA
                            330                           360
GAGGACGGGG AGATTAAGTT TGAAGCAGTC GAGGGTGCAG TAGCGTTGGC GGATCGCGCG
                            390                           420
AGTTCCTTCA TGGTTGACAG CGAGGAATAC AAGATTACGA ACGTAAAGGT TCACGGTATG
                            450                           480
AAGTTTGTCC CAGTTGCGGT TCCTCATGAA TTAAAAGGGA TTGCAAAGGA GAAGTTTCAC
                            510                           540
TTCGTGGAAG ACTCCCGCGT TACGGAGAAT ACCAACGGCC TTAAGACAAT GCTCACTGAG
                            570                           600
GATAGTTTTT CTGCACGTAA GGTAAGCAGC ATGGAGAGCC CGCACGACCT TGTGGTAGAC
                            630                           660
ACGGTGGGTA CCGTCTACCA CAGCCGTTTT GGTTCGGACG CAGAGGCTTC TGTGATGCTG
                            690                           720
AAAAGGGCTG ATGGCTCTGA GCTGTCGCAC CGTGAGTTCA TCGACTATGT GATGAACTTC
                            750                           780
AACACGGTCC GCTACGACTA CTACGGTGAT GACGCGAGCT ACACCAATCT GATGGCGAGT
                            810                           840
TATGGCACCA AGCACTCTGC TGACTCCTGG TGGAAGACAG GAAGAGTGCC CCGCATTTCG
                            870                           900
TGTGGTATCA ACTATGGGTT CGATCGGTTT AAAGGTTCAG GGCCGGGATA CTACAGGCTG
                            930                           960
ACTTTGATTG CGAACGGGTA TAGGGACGTA GTTGCTGATG TGCGCTTCCT TCCCAAGTAC
                            990                          1020
GAGGGGAACA TCGATATTGG GTTGAAGGGG AAGGTGCTGA CCATAGGGGG CGCGGACGCG
                           1050                          1080
GAGACTCTGA TGGATGCTGC AGTTGACGTG TTTGCCGATG GACAGCCTAA GCTTGTCAGC
```

```
                              1110                                    1140
GATCAAGCGG TGAGCTTGGG GCAGAATGTC CTCTCTGCGG ATTTCACTCC CGGCACTGAG
                              1170                                    1200
TACACGGTTG AGGTTAGGTT CAAGGAATTT GGTTCTGTGC GTGCGAAGGT AGTGGCCCAG
                              1230                                    1260
GGATCCAGAC GACGAGGCAG GTCCCCTAGA AGAAGAACTC CCTCGCCTCG CAGACGAAGG
                              1290                                    1320
TCTAAATCGC CGCGTCGCAG AAGATCTCAA TCTCGGGAAT CTCAATGT
                              1350
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 456 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:16 : FROM 1 to 456

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16 :

```
Met Ala Ser Glu Phe Met Gly Ser Ser His His Glu Thr His Tyr Gly
1               5                   10                  15
Tyr Ala Thr Leu Ser Tyr Ala Asp Tyr Trp Ala Gly Glu Leu Gly Gln
            20                  25                  30
Ser Arg Asp Val Leu Leu Ala Gly Asn Ala Glu Ala Asp Arg Ala Gly
        35                  40                  45
Asp Leu Asp Ala Gly Met Phe Asp Ala Val Ser Arg Ala Thr His Gly
    50                  55                  60
His Gly Ala Phe Arg Gln Gln Phe Gln Tyr Ala Val Glu Val Leu Gly
65                  70                  75                  80
Glu Lys Val Leu Ser Lys Gln Glu Thr Glu Asp Ser Arg Gly Arg Lys
                85                  90                  95
Lys Trp Glu Tyr Glu Thr Asp Pro Ser Val Thr Lys Met Val Arg Ala
            100                 105                 110
Ser Ala Ser Phe Gln Asp Leu Gly Glu Asp Gly Glu Ile Lys Phe Glu
        115                 120                 125
Ala Val Glu Gly Ala Val Ala Leu Ala Asp Arg Ala Ser Ser Phe Met
```

```
    130                 135                 140
Val Asp Ser Glu Glu Tyr Lys Ile Thr Asn Val Lys Val His Gly Met
145                 150                 155                 160
Lys Phe Val Pro Val Ala Val Pro His Glu Leu Lys Gly Ile Ala Lys
                165                 170                 175
Glu Lys Phe His Phe Val Glu Asp Ser Arg Val Thr Glu Asn Thr Asn
            180                 185                 190
Gly Leu Lys Thr Met Leu Thr Glu Asp Ser Phe Ser Ala Arg Lys Val
        195                 200                 205
Ser Ser Met Glu Ser Pro His Asp Leu Val Val Asp Thr Val Gly Thr
    210                 215                 220
Val Tyr His Ser Arg Phe Gly Ser Asp Ala Glu Ala Ser Val Met Leu
225                 230                 235                 240
Lys Arg Ala Asp Gly Ser Glu Leu Ser His Arg Glu Phe Ile Asp Tyr
                245                 250                 255
Val Met Asn Phe Asn Thr Val Arg Tyr Asp Tyr Tyr Gly Asp Asp Ala
            260                 265                 270
Ser Tyr Thr Asn Leu Met Ala Ser Tyr Gly Thr Lys His Ser Ala Asp
        275                 280                 285
Ser Trp Trp Lys Thr Gly Arg Val Pro Arg Ile Ser Cys Gly Ile Asn
    290                 295                 300
Tyr Gly Phe Asp Arg Phe Lys Gly Ser Gly Pro Gly Tyr Tyr Arg Leu
305                 310                 315                 320
Thr Leu Ile Ala Asn Gly Tyr Arg Asp Val Val Ala Asp Val Arg Phe
                325                 330                 335
Leu Pro Lys Tyr Glu Gly Asn Ile Asp Ile Gly Leu Lys Gly Lys Val
            340                 345                 350
Leu Thr Ile Gly Gly Ala Asp Ala Glu Thr Leu Met Asp Ala Ala Val
        355                 360                 365
Asp Val Phe Ala Asp Gly Gln Pro Lys Leu Val Ser Asp Gln Ala Val
    370                 375                 380
Ser Leu Gly Gln Asn Val Leu Ser Ala Asp Phe Thr Pro Gly Thr Glu
385                 390                 395                 400
Tyr Thr Val Glu Val Arg Phe Lys Glu Phe Gly Ser Val Arg Ala Lys
                405                 410                 415
Val Val Ala Gln Gly Ser Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg
            420                 425                 430
Thr Pro Ser Pro Arg Arg Arg Arg Ser Lys Ser Pro Arg Arg Arg Arg
        435                 440                 445
Ser Gln Ser Arg Glu Ser Gln Cys
```

450 455

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 153 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:17 : FROM 1 to 153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17 :

```
ATGGCCAGAT ACCGATGCTG CCGCAGCAAA AGCAGGAGCA GATGCCGCCG TCGCAGACGA
                              30                              60
AGATGTCGCA GACGGAGGAG GCGATGCTGC CGGCGGAGGA GGCGAAGATG CTGCCGTCGC
                              90                             120
CGCCGCTCAT ACACCATAAG GTGTAAAAAA TAC
                             150
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: recombinant

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:18 : FROM 1 to 51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18 :

```
Met Ala Arg Tyr Arg Cys Cys Arg Ser Lys Ser Arg Ser Arg Cys Arg
1               5                   10                  15
Arg Arg Arg Arg Arg Cys Arg Arg Arg Arg Arg Arg Cys Cys Arg Arg
            20                  25                  30
```

Arg Arg Arg Arg Cys Cys Arg Arg Arg Arg Ser Tyr Thr Ile Arg Cys
35 40 45

Lys Lys Tyr
50

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 528 nucleic acids

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(vi) ORIGINAL SOURCE:

(A) ORGANISM: synthesized

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Fuminori TAKEMURA et al.

(B) TITLE:

(K) RELEVANT RESIDUES IN SEQ ID NO:19 : FROM 1 to 528

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19 :

ATGGCTAGCA TGAGCACAAA TCCTAAACCT CAAAGAAAAA CCAAACGTAA CACCAACCGC
30 60
CGCCCACGGG ACGTTAAATT CCCGGGCGGT GGTCAGATCG TTGGTGGAGT TTACCTGTTG
90 120
CCGCGCAGGG GCCCCAGGTT GGGTGTGCGC GCGACTAGGA AGACTTCCGA GCGGTCGCAA
150 180
CCTCGTGGAA GGCGACAACC TATCCCCAAG GCTCGCCGGC CCGAGGGTAG GACCTGGGCT
210 240
CAGCCCGGGT ACCCTTGGCC CCTCTATGGC AACGAGGGTA TGGGGTGGGC AGGATGGCTC
270 300
CTGTCACCCC GTGGCTCCCG GCCTAGTTGG GGCCCCACGG ACCCCCGGCG TAGGTCACGC
330 360
AATTTGGGTG AATTCATGGC CAGATACCGA TGCTGCCGCA GCAAAAGCAG GAGCAGATGC
390 420
CGCCGTCGCA GACGAAGATG TCGCAGACGG AGGAGGCGAT GCTGCCGGCG GAGGAGGCGA
450 480
AGATGCTGCC GTCGCCGCCG CTCATACACC ATAAGGTGTA AAAAATAC
510

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 176 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(vi) ORIGINAL SOURCE:
(A) ORGANISM: recombinant
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Fuminori TAKEMURA et al.
(B) TITLE:
(K) RELEVANT RESIDUES IN SEQ ID NO:20 : FROM 1 to 176
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20 :

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Met Ala Ser Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg
1               5                   10                  15
Asn Thr Asn Arg Arg Pro Arg Asp Val Lys Phe Pro Gly Gly Gly Gln
            20                  25                  30
Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly
        35                  40                  45
Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg
    50                  55                  60
Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala
65                  70                  75                  80
Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp
                85                  90                  95
Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro
            100                 105                 110
Thr Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Glu Phe Met Ala Arg
        115                 120                 125
Tyr Arg Cys Cys Arg Ser Lys Ser Arg Ser Arg Cys Arg Arg Arg Arg
    130                 135                 140
Arg Arg Cys Arg Arg Arg Arg Arg Arg Cys Cys Arg Arg Arg Arg Arg
145                 150                 155                 160
Arg Cys Cys Arg Arg Arg Arg Ser Tyr Thr Ile Arg Cys Lys Lys Tyr
                165                 170                 175
```

Claims

1. A nucleic acid-bound polypeptide obtainable by binding a nucleic acid to a polypeptide.

2. The nucleic acid-bound polypeptide as claimed in claim 1, wherein said nucleic acid is bound to at least one terminus of said polypeptide.

3. The nucleic acid-bound polypeptide as claimed in claim 1 or 2, further comprising a nucleic acid-binding motif through which said nucleic acid is bound to said polypeptide.

4. The nucleic acid-bound polypeptide as claimed in claim 3, wherein said polypeptide and said nucleic acid-binding motif are expressed in the form of a fusion polypeptide by genetic engineering.

5. The nucleic acid-bound polypeptide as claimed in claim 3, wherein said nucleic acid-binding motif has the sequence SEQ ID N° 2.

6. The nucleic acid-bound polypeptide as claimed in one of claims 1-5, wherein said polypeptide is an antigen to be used in an immunoassay.

7. A method of producing a nucleic acid-bound polypeptide comprising the steps of :

   producing a polypeptide by genetic engineering,
   binding a nucleic acid to said polypeptide as a soluble fraction, and
   purifying said nucleic acid-bound polypeptide from said soluble fraction.

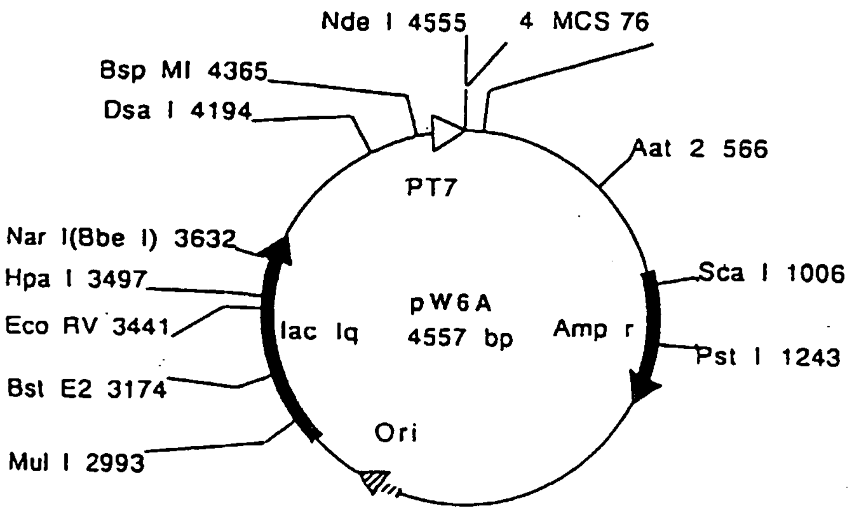
8. The method as claimed in claim 7, wherein the step of binding said nucleic acid to said polypeptide to produce said nucleic acid-bound polypeptide comprises the steps of :

   fusing a gene which encodes said polypeptide and a gene which encodes a nucleic acid-binding motif for binding said nucleic acid to said polypeptide to produce a fusion gene, and
   expressing said fusion gene to produce said nucleic acid-bound polypeptide via said nucleic acid-binding motif.

9. An immunoassay for assaying an antigen comprising a polypeptide, or an antibody corresponding to said antigen, which comprises using a nucleic acid-bound polypeptide as claimed in one of claims 1-5, obtainable by binding a nucleic acid to the polypeptide of said antigen.

10. An agglutination immunoassay for assaying an antigen comprising a polypeptide, or an antibody corresponding to said antigen, which comprises using a nucleic acid-bound polypeptide as claimed in one of claims 1-5, obtainable by binding a nucleic acid to the polypeptide of said antigen, said nucleic acid-bound polypeptide being fixed on the surface of particles.

Fig. 1



4475 4495 0

TAATACGACTCACTATATGGAATTGTGAGCGGATAACAATT......................CATATG

T7 promoter lac operator Nde I

| Nhe I | | Eco RI | | Sal I | | Xho I | | Bam HI | | Apa I | | Xba I |

MCS: ATG GCT AGC GAA TTC GTC GAC CTC GAG GGA TCC GGG CCC TCT AGA

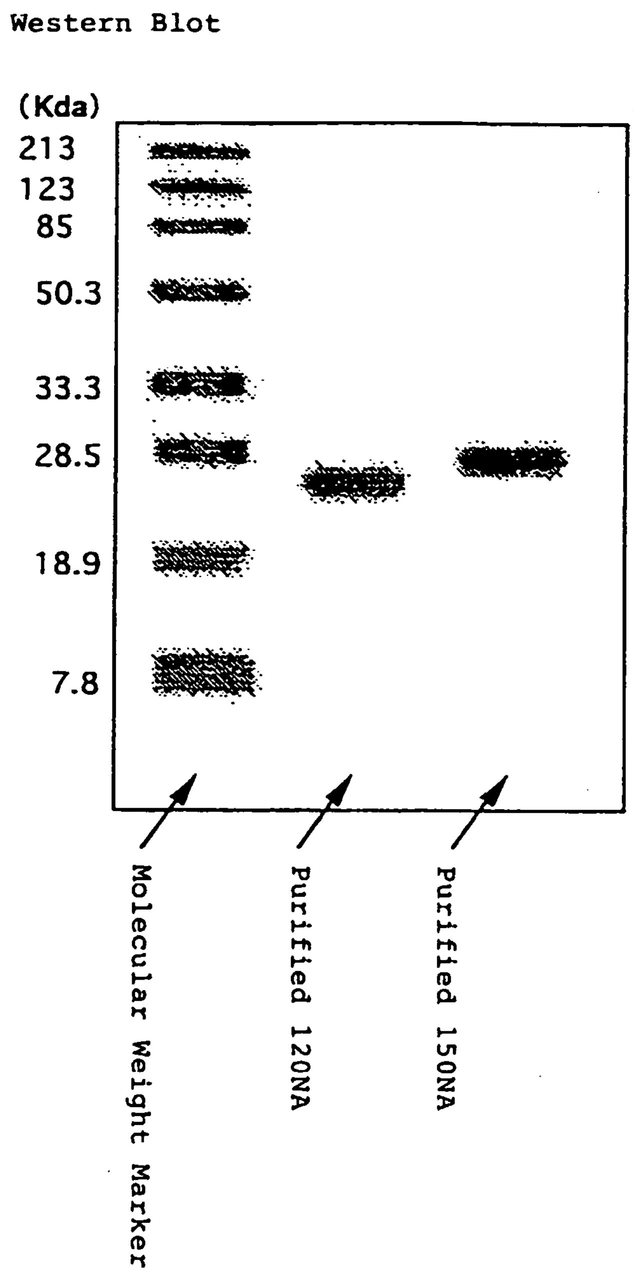
start codon

| Not I | | Eco T22I | | Kpn I | | | Hind 3 |

TGC GGC CGC ATG CAT GGT ACC TAA CTA ACT AAG CTT

Eag I TL term

Fig. 2


Western Blot
(Kda)
213
123
85
50.3
33.3
28.5
18.9
7.8
Molecular Weight Marker
Purified 120NA
Purified 150NA

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 97400985.4

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 6)
X	WO - A - 93/14 768 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) * Abstract * --	1,3,7, 8	C 07 K 2/00 C 12 N 15/62 G 01 N 33/53
X	EP - A - 0 704 221 (AJINOMOTO CO., INC.) * Abstract * --	1,7	
X,D	JOURNAL OF VIROLOGY, vol. 64, no. 7, July 1990 F. BIRNBAUM et al. "Hepatitis B Virus Nucleocapsid Assembly: Primary Structure Requirements in the Core Protein" pages 3319-3330 * Page 3319 * ----	1,3	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 6)
			C 07 K C 12 N G 01 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	06-08-1997	WOLF

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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